Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2020.0606.01**

THIAZOLE CHALCONES INTERACTION WITH TUBULIN

Teodora Constantinescu[1], Claudiu N. Lungu[2,3]*, Ovidiu Oniga[1]

1. Department of Chemistry, Pharmaceutical Chemistry, Faculty of Pharmacy, Iuliu Hatieganu University, 400012 Cluj Romania.

2. Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, 400028 Cluj, Romania.

3. Surgery Department, County Emergency Hospital, Braila, Romania.

ABSTRACT: Tubulin is a target for anticancer therapy. Chalcones are antimitotic compounds that inhibit the polymerization of tubulin by binding to colchicine site. Thiazole is a heterocycle with anticancer properties. Studies in silico conducted show that thiazolic chalcones show no mass effect, and ligand-receptor energy is low. From 20 thiazole chalcones, four compounds show good binding ability. Results were compared with 31 α-methyl chalcone taken from literature. The specificity of thiazole chalcones is superior.

Keywords: Binding affinity, chalcone, thiazole, tubulin.

Article History: Received: September 28, 2020; Revised: October 14, 2020; Accepted: November 01, 2020.

Corresponding Author: Claudiu N. Lungu*

1. Department of Chemistry, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, 400028 Cluj, Romania.

2. Surgery Department, County Emergency Hospital, Braila, Romania.

Email address:lunguclaudiu5555@gmail.com

1.INTRODUCTION

Microtubules are vital cytoscheletic filaments of great importance in mitosis and cell division processes, being an important therapeutic target for anticancer therapy [1]. They are involved in a series of cellular processes, including motility, cell signaling, cell shape maintenance, intracellular proliferation, and transport [2,3]. The essential component of microtubules is tubulin [4]. The dynamics of assembling and disassembly processes of microtubules is a vital target to identify new

anticancer agents [5]. Tubulin polymerization takes place through a nucleation elongation mechanism, which is completed by reversible, covalent addition of tubulin dimers. At microtubule level, active compounds bind to one of three main binding domains of paclitaxel, vinca alkaloids, and colchicine class [6,7]. Tubulin ligands are compounds that inhibit the formation of the spindle (e.g., colchicines, vinblastine, vincristine) or compounds that inhibit spindle formed (e.g., paclitaxel, docetaxel) [4]. Inhibition of tubulin polymerization or interference with microtubule disassembly results in a cell cycle arrest and induces apoptosis [1]. Antivascular effects of anti-tubulin compounds result from the role of tubulin and microtubules in determining the elongation form of vascular endothelial cells. Cell microtubule network, a major part of cytoskeleton, has a significant role in maintaining cell shape, particularly in neovascularization. Endothelial cells of immature vasculature have less developed cytoskeleton actin, which is the reason why they are more sensitive to effects of antitubulinic agents [8]. Clinical use of these compounds is limited by various aspects such as increased toxicity, resistance to therapy, numerous adverse effects, low oral solubility, and bioavailability, and complex synthesis [9,10]. An essential feature of anticancer therapy is the selective action of biologically active agents on malignant cells. Many therapeutic agents act on both malignant and normal cells. In case of cancer cells, abnormal proliferation, ability to form metastasis, and the need for apoptosis provide a continuing challenge for identifying new therapeutic agents [4]. Chalcones are flavonoids in which two aromatics units are joined by an α, β-unsaturated carbonyl system (1,3-diphenyl-2-propen-1-one) [11]. E configuration is more thermodynamically stable [12]. They are usually synthesized by Claisen-Schmidt condensation reaction of acetophenones with arylaldehydes in acidic or basic catalysis [13,14]. Due to interconversion of chalcones in presence of acids and bases, they are important ligands [15]. Natural and synthetic chalcones have anti-inflammatory, antidiabetic, antiviral, anti-Alzheimer, antimicrobial, anti-fungal, antioxidant, gastric protectant, antiangiogenic anticancer activities [3,10,16-23]. Natural chalcones are key structures with many biological activities (e.g., curcumin, xanthohumol, isoliquiritigenin, flavokawain), which have a particular interest in identification of new anticancer agents [24,25]. It is known that some chalcones substituted on aromatic nuclei have cytotoxic and antimitotic activity due to their ability to inhibit binding to tubulin and inhibits microtubule polymerization. These compounds show this effect due to their reversible binding to the colchicine binding site [26,27]. Most natural anti-tubulin chalcones are substituted with hydroxy and methoxy groups in different positions of two aromatic residues [28]. Other mechanisms of action of chalcones are inhibition of angiogenesis, induction of apoptosis, antiestrogenic activity, and reversal of resistance to therapy. Chalcones can act by one or more mechanisms [29]. Millepachine, a chalcone with a 2,2-dimethylbenzopyran subunit, exhibits significant cytotoxicity *in vitro* on various types of cancer cells and intense antitumoral activity *in vivo* [2]. Methoxychalcones are structurally similar to combretastatin A4-5 and colchicine due to similar spatial orientation between aldehyde and

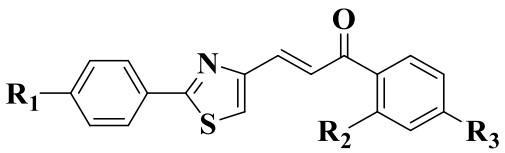
acetophenone. SAR studies have shown that antitubulinic properties of methoxychalcones are dependent on substituents from 2, 4, and 6 positions of aldehyde [29]. Konieczny et al. have synthesized oxazole chalcones and have shown that (E) -1- (benzo [d] [1,3] oxathiol-6-yl) -3-phenylprop-2-en-1-one exhibits high cytotoxicity at nanomolecular level, which can be attributed tocombined influence of three structural factors: 1) presence of a heterocyclic ring, 2) the presence of a 5-OR residue on acetophenone and 3) substituents from aldehyde [30]. From pentatomic heterocycles, thiazole is a crucial part of medical chemistry [31]. This nucleus is an essential component of a large number of therapeutic agents with anticancer, anticonvulsant, antifungal, and antibacterial properties [32]. Compounds of a series of 4-substituted methoxybenzoyl-aryl-thiazole inhibit tubulin polymerization by binding to colchicine site. A phenylaminothiazole derivative of series, (2-((4-fluorophenyl)amino) thiazole-4-yl)(3,4,5-trimethoxyphenyl) methanone, inhibits tubulin polymerization with an increased potency at nanomolar range [33]. The purpose of the study is to analyze binding capacity and specificity for newly synthesized 20 thiazole chalcones and to compare obtained results with a similar study conducted by our team for 31 α-methylcalcones taken from literature.

2. MATERIALS AND METHODS

Tubulin -BAL27862 complex (PDB ID 4O2A) retrieved from literature as a target for thiazole chalcones, was modeled computationally using the AMBER 94 force field. A set of 20 thiazole chalcones were prepared in silico using the MM2 force field.

The structure of thiazole chalcone is represented in **Table 1**.

Table 1: Structure of thiazole chalcones



a-t

a-t	R_1	R_2	R_3	Name
a	H	H	OH	(E)-1-(4-hydroxyphenyl)-3-(2-phenylthiazol-4-yl)prop-2-en-1-one
b	CH_3	H	OH	(E)-1-(4-hydroxyphenyl)-3-(2-(p-tolyl)thiazol-4-yl)prop-2-en-1-one
c	Cl	H	OH	(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(4-hydroxyphenyl)prop-2-en-1-one

d	OCH_3	H	OH	(E)-1-(4-hydroxyphenyl)-3-(2-(4-methoxyphenyl)thiazol-4-yl) prop-2-en-1-one
e	H	OH	H	(E)-1-(2-hydroxyphenyl)-3-(2-phenylthiazol-4-yl)prop-2-en-1-one
f	CH_3	OH	H	(E)-1-(2-hydroxyphenyl)-3-(2-(p-tolyl)thiazol-4-yl)prop-2-en-1-one
g	Cl	OH	H	(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one
h	OCH_3	OH	H	(E)-1-(2-hydroxyphenyl)-3-(2-(4-methoxyphenyl)thiazol-4-yl) prop-2-en-1-one
i	H	H	OCH_3	(E)-1-(4-methoxyphenyl)-3-(2-phenylthiazol-4-yl)prop-2-en-1-one
j	CH_3	H	OCH_3	(E)-1-(4-methoxyphenyl)-3-(2-(p-tolyl)thiazol-4-yl)prop-2-en-1-one
k	Cl	H	OCH_3	(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(4-methoxyphenyl) prop-2-en-1-one
l	OCH_3	H	OCH_3	(E)-1-(4-methoxyphenyl)-3-(2-(4-methoxyphenyl)thiazol-4-yl) prop-2-en-1-one
m	H	OCH_3	OCH_3	(E)-1-(2,4-dimethoxyphenyl)-3-(2-phenylthiazol-4-yl)prop-2-en-1-one
n	CH_3	OCH_3	OCH_3	(E)-1-(2,4-dimethoxyphenyl)-3-(2-(p-tolyl)thiazol-4-yl) prop-2-en-1-one
o	Cl	OCH_3	OCH_3	(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one
p	OCH_3	OCH_3	OCH_3	(E)-1-(2,4-dimethoxyphenyl)-3-(2-(4-methoxyphenyl)thiazol-4-yl)prop-2-en-1-one
q	H	OCH_3	H	(E)-1-(2-methoxyphenyl)-3-(2-phenylthiazol-4-yl)prop-2-en-1-one
r	CH_3	OCH_3	H	(E)-1-(2-methoxyphenyl)-3-(2-(p-tolyl)thiazol-4-yl)prop-2-en-1-one
s	Cl	OCH_3	H	(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(2-methoxyphenyl) prop-2-en-1-one
t	OCH_3	OCH_3	H	(E)-1-(2-methoxyphenyl)-3-(2-(4-methoxyphenyl)thiazol-4-yl) prop-2-en-1-one

Ligands were docked against tubulin (4O2A) using AutoDock software [34]. Binding sites were retrieved from literature and using a molecular surface (Van der Waals algorithm). The number of cavities was set to 4. Binding energies were computed. The sum of total energy was computed. Four cavities were chosen to study the most promising compounds in terms of the energy of binding.

Results

Cavities obtained were as follow : Cavity A (1561.09 x 15.93 y44.26, z9.85), cavity B (974.336 x 17.73, y 67.99, z84.49), cavity C (508.928 x 13.20,y 21.08, z -20.90), Cavity D (318.976x 14.06,y 75.62, z44.71). Docking energyes (total energy) for each ligand are represented in **Table 2.**

Table 2. Binding energies of ligands for distinct binding sites (A, B, C, D) and some of binding sites energys or each distinct ligands (A+B+C+D)

Compound	**Cavity A**	**Cavity B**	**Cavity C**	**Cavity D**	**A+B+C+D**
a	-122.538	-112.603	-116.404	-111.906	-463.45
b	-125.021	-114.318	-122.429	-115.966	-477.734
c	-121.209	-111.676	-111.778	-106.828	-451.491
d	-135.398	-119.32	-129.342	-100.999	-485.059
e	-123.046	-108.734	-125.897	-106.944	-464.621
f	-110.075	-112.003	-116.77	-106.607	-445.455
g	-111.484	-118.583	-114.06	-118.031	-462.158
h	-127.953	-116.833	-126.016	-116.786	-487.588
i	-122.691	-110.671	-110.893	-109.293	-453.548
j	-124.807	-126.97	-125.208	-118.908	-495.893
k	-130.008	-122.334	-122.11	-109.739	-484.191
l	-126.817	-112.493	-134.354	-125.45	-499.114
m	-131.795	-96.1689	-147.198	-112.146	-487.3079
n	-135.802	-123.936	-123.468	-119.002	-502.208
o	-133.552	-127.615	-125.324	-107.911	-494.402
p	-128.727	-113.641	-131.508	-128.933	-502.809
q	-96.1527	-100.039	-119.993	-104.429	-420.6137
r	-108.45	-116.133	-129.423	-99.2839	-453.2899
s	-123.262	-109.968	-138.559	-107.608	-479.397
t	-122.81	-120.501	-140.429	-102.452	-486.192

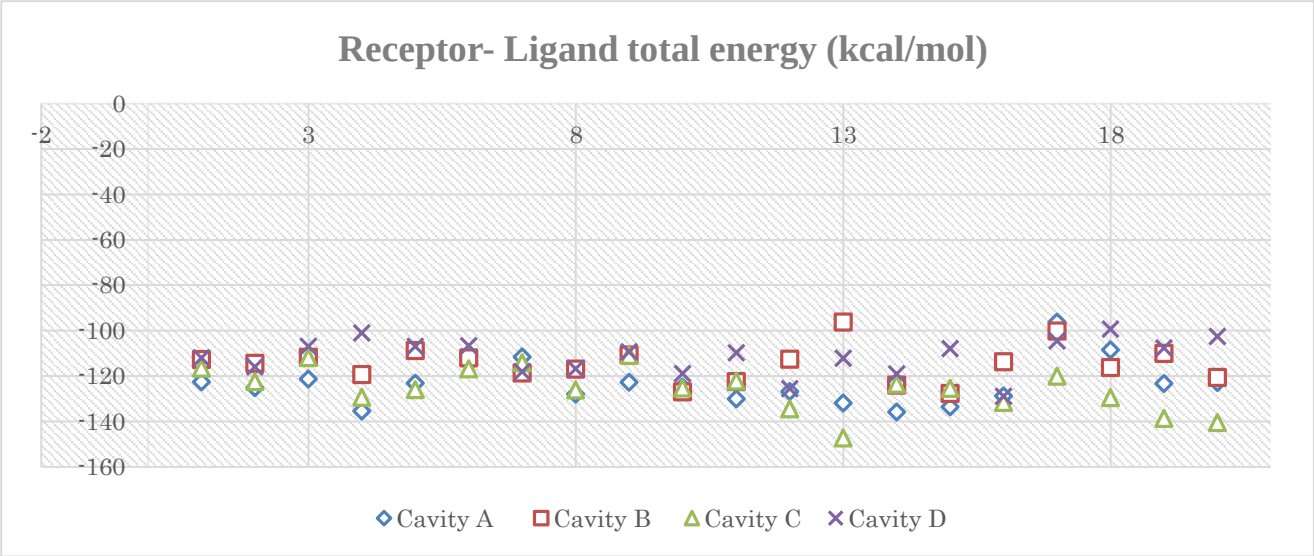
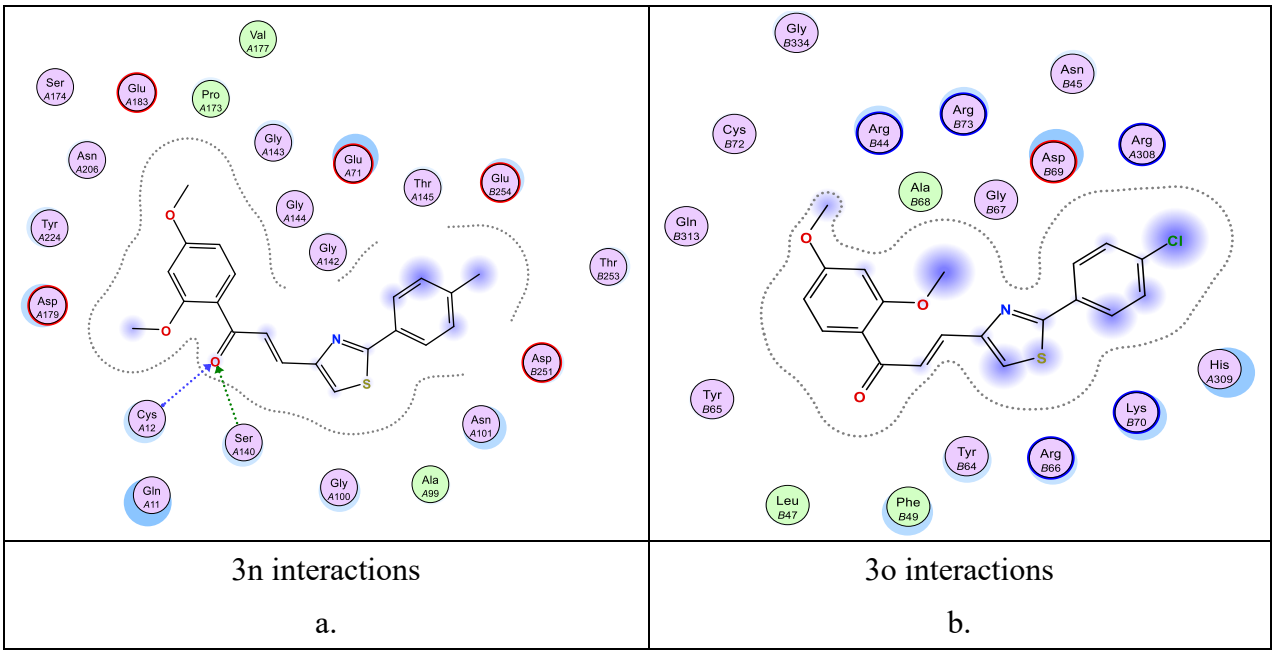
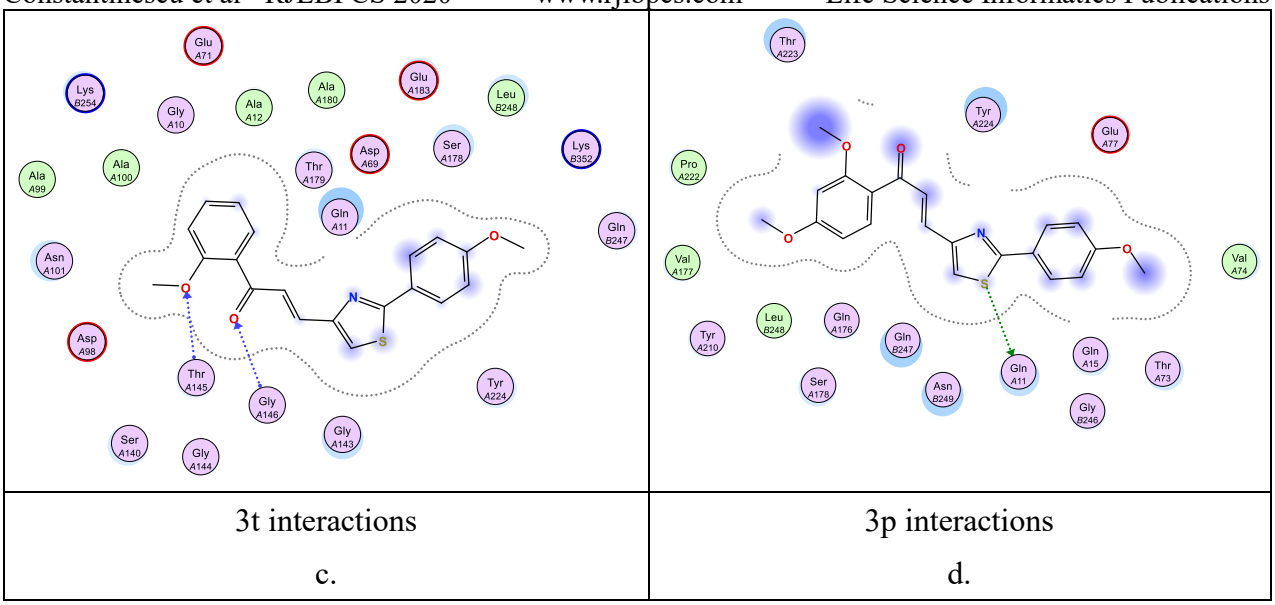


Figure 2: Receptor-ligands total energy (kcal/mol) is represented. The energy has some fluctuations for four binding sites (cavity 1 to 4). Also, the lowest energies are observed in the case of binding site C and higher values for binding site B. In some cases, there are some overlap regions with slightly similar values for all four binding sites (cavities).



3n interactions	3o interactions
a.	b.



3t interactions	3p interactions
c.	d.

Figure 3: Ligand interactions with tubulin binding sites.

Ligand 3n interacts with tubulin by a side chain acceptor (Cys A12) and a side chain donor (Ser A140) (**Fig. 3a**). Ligand 3o interacts with tubulin binding sites by two methyl groups exposed to solvent a heterocyclic and a halogen atom (Cl) expose to solvent, respectively. (**Fig. 3b**). Ligand 3t interacts with tubulin binding sites by to oxygen atoms that form a backbone acceptor bound with Thr A145, and Gly A146, some ligand exposer to solvent is also involved (**Fig. 3c**). Ligand 3p interacts with tubulin by side-chain acceptor bound between heterocyclic sulfur and Gln A11; three methyl groups, one heterocyclic nitrogen, and a keto group are exposed to the solvent (**Fig. 3d**).

3. RESULTS AND DISCUSSION

The affinity of a ligand to a receptor depends mainly on their ability to form a ligand-receptor complex. Complex formation is an active process involving numerous intermolecular interactions that stabilize and destabilize the complex formation process [35]. Interactions of proteins with other molecules are essential for defining their functionality. Such interactions are involved in all processes in biological systems, but also in external modulation of proteins by external agents (e.g., drugs) [36]. Identifying new molecules that bind strongly and specifically to molecular targets is an essential goal for many areas of molecular science [37]. Rational evaluation of new medicinal agents is facilitated by understanding how small molecules interact with macromolecular targets [38]. In silico studies are used as pre-selective elements aimed at accelerating identification processes of new drugs by reducing the number of ligands that can be synthesized and analyzed for their effectiveness and mode of action [39]. Analysis of the formation of ligand-receptor complexes is a preliminary stage particularly important for studies [40]. Identification of binding receptor proteins for ligands indicates valuable information for signal transduction and drug action [41]. Biological activities of chalcones are attributed to α,β-unsaturated ketone subunit. Study of these compounds is due to facile synthesis, simple chemical architecture and possibility of these to be precursors to

numerous biologically active molecules. One of the most proposed mechanisms of anticancer activity for these compounds is the inhibition of tubulin polymerization by binding to cholchicine site [42]. Due to potential anticancer activities, identification of new chalcones has been much studied. Most of these natural compounds with anticancer properties are substituted with electron donor groups (hydroxy or/and methoxy) in different positions of the base structure [43]. Edwards has demonstrated that the presence of a subunit of trimethoxyphenyl in chalcone is responsible for irreversible inhibition of tubulin. This is due to interaction with the rest of cysteine from tubulin, which binds to a Michael-type addition [44]. Polymethoxylated chalcones on aromatic subunits show activity on human leukemic cell lines K562 at nanomolar concentrations. Action is due to inhibition of mitosis and inhibition of tubulin polymerization. The ability to block cells in the G2/M phase of cell cycle is correlated with property to inhibit tubulin polymerization [45]. On the other hand, 4-amino-5-benzoyl-2-(4-methoxyphenylamino)thiazole exhibits cytotoxic properties on different cell lines. Effects are related to blocking mitosis and disrupting the division spindle of mitotic cells. It also inhibits the assembly of microtubular proteins by binding of colchicine site to tubulin [46].

Results obtained show that receptor-ligands energy differs for the four binding sites (Fig.2)

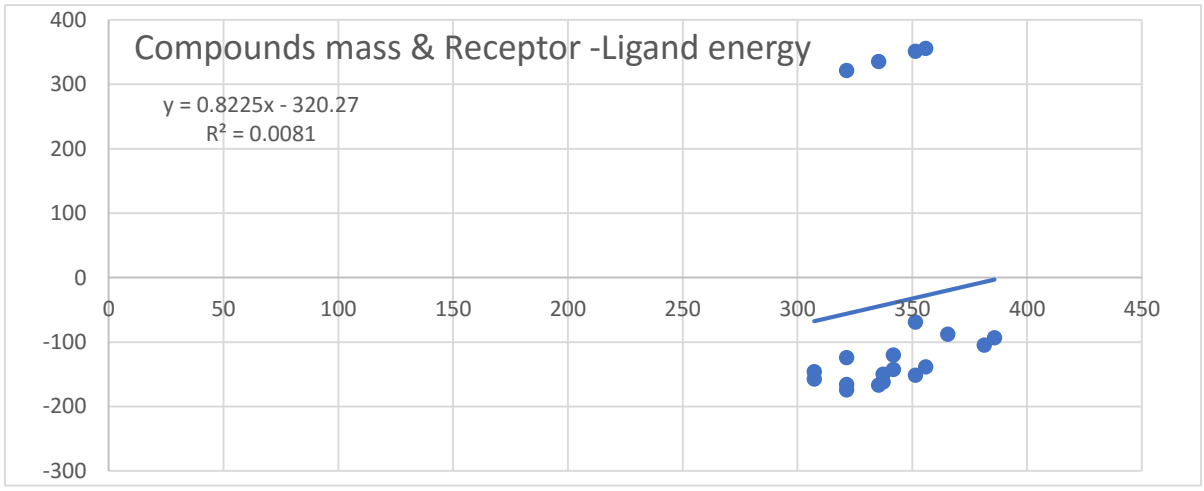


Figure 4: Correlation between molecular mass and total energy

Figure 4 shows that thiazole chalcone has no mass effect, as ligand - mass and energy show no correlation (r^2=0.0081)

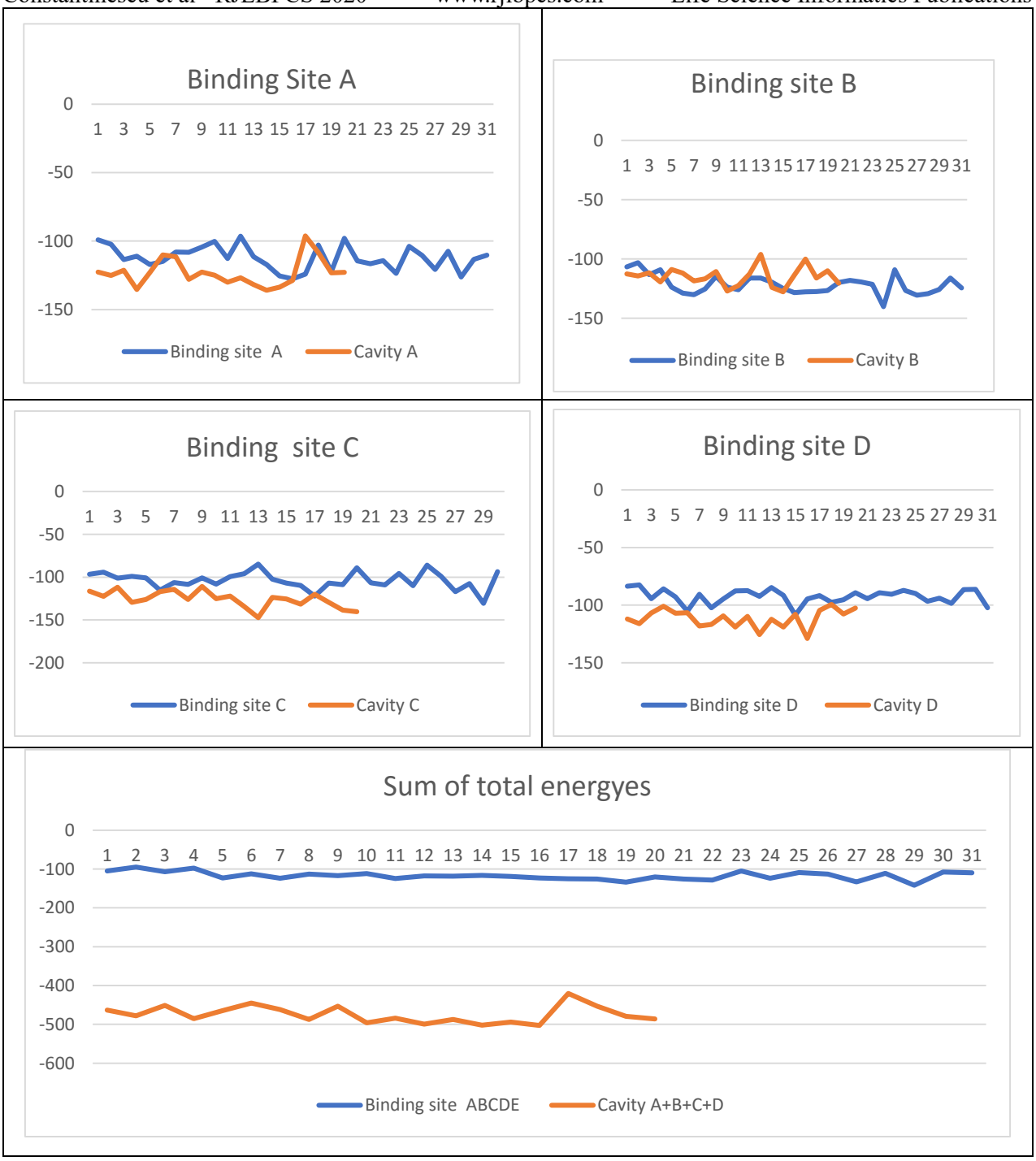


Figure 5: Energies of 20 thiazole chalcones (orange) compared to 31 α-methylcalcones (blue)

Results for 20 thiazoles chalcones analyzed and previously synthesized by our team were compared with a study we previously conducted for 31α-methylcalcones with anticancer potential taken from literature (**Fig. 5**). It is noted that both thiazole and α-methylcalcone are devoid of specificity. In the case of thiazole chalcones, specificity is better, which is an essential advantage compared to the other series analyzed. Four thiazole compounds have the best binding capacity (n,o,p, and t). Results show that substitution with methoxy groups on thiazole aldehyde and acetophenone is favorable for binding ability. For methylcalcone, the best binding capacity presents compound with a hydroxy

group in position 4 of acetophenone and rest of dimethylamine in para position of aldehyde [47, 48].

4. CONCLUSION

Interactions with tubulin for 20 thiazoles previously obtained by our team were analyzed. Chalcones have no mass effect, and ligand-receptor energy is low. Substitution with methoxy groups is favorable for the binding affinity of thiazole chalcones. The predicted specificity of analyzed compounds is low. Compared to 31 α-methyl chalcone previously studied, thiazole chalcones show lower ligand-receptor energies and better specificity.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

FUNDING

None

ACKNOWLEDGEMENT

All authors contributed equally to this manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Sharma S, Kaur C, Budhiraja A, Nepali K, Gupt M. Saxena AK, Bedi PMS, Chalcone based azacarboline analogues as novel antitubulin agents: Design, synthesis, biological evaluation and molecular moddleing studies, Eur J Med Chem. 2014; 85: 648-660;
2. Huang X, Huag R, Li L, Gou S, Wang H, Synthesis and biological evaluation of novel chalcone derivatives as new class of microtubule destabilizing agents, Eur J Med Chem. 2017; 132: 11-25;
3. Huang X, Huang R, Wang Z, Li L, Gou S, Liao Z, et al. Pt(IV) complexes conjugating with chalcone analogue as inhibitors of microtubule polymerization exhibited selective inhibition in human cancer cells. Eur J Med Chem 2018;146:435–50. Available from:
4. Mirzaei H, Emami S, Recent advances od cytotoxic chalconoids targeting tubulin polymerization: Synthesis and biological activity, Eur J Med Chem. 2016; 121: 610-639;

5. Sultana F, Reddy Bonam S, Reddy VG, Nayak VL, Akunuri R, Rani Routhu S, et al. Synthesis of benzo[d]imidazo[2,1-b]thiazole-chalcone conjugates as microtubule targeting and apoptosis inducing agents. Bioorg Chem 2018;76:1–12.
6. Xu F, Li W, Shuai W, Yang L, Bi Y, Ma C, et al. Design, synthesis and biological evaluation of pyridine-chalcone derivatives as novel microtubule-destabilizing agents. Eur J Med Chem. 2019;173:1–14.
7. Ducki S, Mackenzie G, Greedy B, Armitage S, Chabert JFD, Bennett E, et al. Combretastatin-like chalcones as inhibitors of microtubule polymerisation. Part 2: Structure-based discovery of alpha-aryl chalcones. Bioorganic Med Chem 2009;17(22):7711–22.
8. Ducki S, Rennison D, Woo M, Kendall A, Chabert JFD, McGown AT, et al. Combretastatin-like chalcones as inhibitors of microtubule polymerization. Part 1: Synthesis and biological evaluation of antivascular activity. Bioorganic Med Chem 2009;17(22):7698–710.
9. Wang G, Qiu J, Xiao X, Cao A, Zhou F. Synthesis, biological evaluation and molecular docking studies of a new series of chalcones containing naphthalene moiety as anticancer agents. Bioorg Chem 2018;76:249–57.
10. Li W, Yin Y, Yao H, Shuai W, Sun H, Xu S, et al. Discovery of novel vinyl sulfone derivatives as anti-tumor agents with microtubule polymerization inhibitory and vascular disrupting activities. Eur J Med Chem [Internet]. 2018;157:1068–80.
11. Evanghelista F, Bandeira M, Silva G, Silva M, Andrade S, Marques D et al, Synthesis and in vitro evaluation of novel triazole/azide chalcone, Med Chem Res., 2016, 26: 27-43;
12. Marquina S, Maldonado-Santiago M, Sánchez-Carranza JN, Antúnez-Mojica M, González-Maya L, Razo-Hernández RS, et al. Design, synthesis and QSAR study of 2′-hydroxy-4′-alkoxy chalcone derivatives that exert cytotoxic activity by the mitochondrial apoptotic pathway. Bioorganic Med Chem 2019; 27(1):43–54.
13. Reddy VB, Su CR, Ciou WF, Liu YN, Chen RY, Bastov K et al, Design, synthesis and biological evaluation of Mannich bases of heterocyclic chalcone analogues as cytotoxic agents, Bioorg Med Chem, 2008, 16: 7358-7370;
14. Constantinescu T, Leonte D, Bencze LC, Vlase L, Imre S, Hanganu D, et al. Heterocycles 43. Synthesis, characterization and antioxidant activity of some thiazole hydroxychalcones and their flavonoidic derivatives. Farmacia. 2018;66(4):663–73.
15. Mahapatra DK, Bharti SK, Asati V, Singh SK. Perspectives of medicinally privileged chalcone based metal coordination compounds for biomedical applications. Eur J Med Chem 2019;174:142–58.
16. Riaz S, Iqbal M, Ullah R, Zahra R, Chotana GA, Faisal A, et al. Synthesis and evaluation of novel α-substituted chalcones with potent anticancer activities and ability to overcome multidrug resistance. Bioorg Chem 2019; 87: 123–35.

17. Han X, Peng B, Xiao BB, Cao SL, Yang CR, Wang WZ, et al. Synthesis and evaluation of chalcone analogues containing a 4-oxoquinazolin-2-yl group as potential anti-tumor agents. Eur J Med Chem 2019; 162:586–601.
18. Shankaraiah N, Nekkanti S, Brahma UR, Praveen Kumar N, Deshpande N, Prasanna D, et al. Synthesis of different heterocycles-linked chalcone conjugates as cytotoxic agents and tubulin polymerization inhibitors. Bioorganic Med Chem 2017; 25(17): 4805–16.
19. Chidan Kumar CS, Loh WS, Ooi CW, Quah CK, Fun HK. Structural correlation of some heterocyclic chalcone analogues and evaluation of their antioxidant potential. Molecules. 2013;18(10):11996–2011.
20. Xu M, Wu P, Shen F, Ji J, Rakesh KP. Chalcone derivatives and their antibacterial activities: Bioorg Chem 2019; 19: 103133.
21. Detsi A, Majdalani M, Kontogiorgis CA, Hadjipavlou-Litina D, Kefalas P. Natural and synthetic 2′-hydroxy-chalcones and aurones: Synthesis, characterization and evaluation of the antioxidant and soybean lipoxygenase inhibitory activity. Bioorganic Med Chem 2009;17(23):8073–85.
22. Wu JZ, Cheng CC, Shen LL, Wang ZK, Wu SB, Li WL, et al. Synthetic chalcones with potent antioxidant ability on H<inf>2</inf>O<inf>2</inf>-induced apoptosis in PC12 cells. Int J Mol Sci. 2014;15(10):18525–39.
23. Wang Y, Hedblom A, Koerner SK, Li M, Jernigan FE, Wegiel B, et al. Novel synthetic chalcones induce apoptosis in the A549 non-small cell lung cancer cells harboring a KRAS mutation. Bioorganic Med Chem Lett. 2016;26(23):5703–6.
24. Takac P, Kello M, Pilatova MB, Kudlickova Z, Vilkova M, Slepcikova P, et al. New chalcone derivative exhibits antiproliferative potential by inducing G2/M cell cycle arrest, mitochondrial-mediated apoptosis and modulation of MAPK signalling pathway. Chem Biol Interact 2018; 292(May):37–49.
25. Wintee C, Caetano JN, Aranjo ABC, Chaves AR, Ostroski IC, Vaz BG et al, Activated carbons for chalcone production: Claisen-Schmidt condensation reaction, Chem Eng J. 2016; 303: 604-610;
26. Kumar D, Maruthi Kumar N, Tantak MP, Ogura M, Kusaka E, Ito T. Synthesis and identification of α-cyano bis(indolyl)chalcones as novel anticancer agents. Bioorganic Med Chem Lett 2014;24(22):5170–4.
27. Dyrager C, Wickström M, Fridén-Saxin M, Friberg A, Dahlén K, Wallén EAA, et al. Inhibitors and promoters of tubulin polymerization: Synthesis and biological evaluation of chalcones and related dienones as potential anticancer agents. Bioorganic Med Chem. 2011;19(8):2659–65.
28. Mesenzani O, Massarotti A, Giustiniano M, Piroli T, Bevilacqua V, Caldarelli A et al, Replacement of the double bond of antitubuline chalcones with triazoles and tetrazoles: Synthesis and biological evaluation, Bioorg Med Chem Lett., 2011, 21: 764-768;

29. Pawar C, Sarkate A, Karnik K, Bahekar S, Pansare D, Shelke R et al, Synthesis and antimicrobial evaluation of novel ethyl 2-(2-(4-substituted)acetamido)-4-substituted-thiazole-5-carboxylate derivatives, Bioorg Med Chem Lett, 2016, 26: 3525-3258.
30. Konieczny MT, Bułakowska A, Pirska D, Konieczny W, Skladanowski A, Sabisz M, et al. Structural factors affecting affinity of cytotoxic oxathiole-fused chalcones toward tubulin. Eur J Med Chem. 2015;89(2):733–42.
31. Nastasa C, Tiperciuc B, Duma M, Benedec D, Oniga O. New hydrazones bearing thiazole scaffold: Synthesis, characterization, antimicrobial, and antioxidant investigation. Molecules. 2015;20(9):17325–38.
32. Pawar C, Sarkate A, Karnik K, Bahekar S, Pansare D, Shelke R et al, Synthesis and antimicrobial evaluation of novel ethyl 2-(2-(4-substituted)acetamido)-4-substituted-thiazole-5-carboxylate derivatives, Bioorg Med Chem Lett, 2016, 26: 3525-3258.
33. Guggilapu SD, Guntuku L, Reddy TS, Nagarsenkar A, Sigalapalli DK, Naidu VGM et al. Synthesis of thiazole linked indolyl-3-glyoxylamide derivatives as tubulin polymerization inhibitors. Eur J Med Chem 2017;138:83–95.
34. http://autodock.scripps.edu/resources/references
35. Rojas S, Parravicini O, Vettorazzi M, Tosso R, Garro et al. Combined MD/QTAIM techniques to evaluate ligand-receptor interactions. Scope and limitations. Eur J Med Chem 2020, 208,112792.
36. Becker W, Bhattiprolu KC, Gubensäk N, Zangger K. Investigating protein-ligand interaction bysolution nuclear magnetic resonance spectroscopy. ChemPhysChem 2018; 19:895-906.
37. Kangas E. Optimization electrostatic affinity in ligand-receptor binding: Theory, computation, and ligand properties. J Chem Phys 1998; 109(17): 7522-7544.
38. Williamson PTF. Solid-state NMR for the analysis of high-affinity ligand/receptor interactions. Concepts Magn Reson 2009; 34A(3): 144-172.
39. Krammer A, Kirchhoff P, Jiang X, Venkatachalam CM, Waldman M. LigScore: a novel scoring function for predicting binding affinities. J Mol Graph Model 2005; 23(5):395-407.
40. Kulbatskii DS, Shulepko MA, Sluchanko NN, YablokovEO, Kamyshinsky RA et al. Efficient screening of ligand-receptor complex formation using fluorescence labeling and size-exclusion chromatography. Biochem Biophys Res Comm 2020, 532: 127-133.
41. Lopez-Garcia L, Demiray L, Ruch-Marder S,Hopp AK, Hottiger M et al. Validation of extracellular ligand-receptor interaction byFlow-TriCEPS. BMC Res Notes 2018; 11: 863
42. Shankaraiah N, Nekkanti S, Brahma UR, Kumar NP, Deshpande N et al. Synthesis of different heterocycles-linked chalcone conjugates as cytotoxic agents and tubulin polymerization inhibitors. Bioorg Med Chem 2017; 25: 4805-4816.

43. Shankaraiah N, Nekkanti S, Brahma UR, Kumar NP, Deshpande N et al. Synthesis of different heterocycles-linked chalcone conjugates as cytotoxic agents and tubulin polymerization inhibitors. Bioorg Med Chem 2017; 25: 4805-4816.
44. Mirzaei S, Hadizadeh F, Eisvand F, Mosaffa F, Ghodsi R. Synthesis, structure-activity relationship and molecular docking studies of novel quinoline-chalcone hybrids as potential anticancer agents and tubulin inhibitors. J Mol Struct 2020; 1202:127310.
45. Ducki S. Antimitotic chalcones and related compounds as inhibitors of tubulin assembly. Anticancer Agents Med Chem 2009; 9(3): 336-347.
46. Sengupta S, Smitha S, Thomas N, Santhoshkumar T, Devi S et al. 4-Amino-5-benzoyl-2-(4-methoxy-phenylamino)thiazole (DAT1): a cytotoxic agent towards cancer cells and a probe for tubulin-microtubule systen. British J Pharmacol 2005;14:1076-1083.
47. Constantinescu T, Leonte D, Bencze LS, Vlase L, Imre S et al. Heterocycles 43. Synthesis, characterization and antioxidant activity of some thiazole hydroxychalcones and their flavonoidic derivatives. Farmacia 2018; 66(4): 663-673.
48. Constantinescu T, Lungu C. Chalcones lack of specificity: Tubuline, a multitarget molecule. RJLBPCS 2018; 4(5): 260-268.